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Association Between Climatic Factors with Fresh Semen Quality Parameters in Madura Cattle (an Indonesian Native Breed)

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Abstract: Semen quality is a critical point to ensure the success of the artificial insemination program. One factor which highly determine the semen quality of bulls is climatic conditions. The purpose of the present study was to evaluate the association between climatic factors and fresh semen quality parameters in Madura bulls. A total of 1583 ejaculates which were collected from 5 Madura bulls from January 2014 to December 2018 were included in this study. Semen quality parameters such as semen Volume (VOL), sperm Concentration (CONC), Total sperm (TOT), sperm Motility (MOT) and Straw production (STR) were directly evaluated upon collection and then correlated with climate factors such as mean and maximum ambient temperature (AT_{mean} and AT_{max}, respectively), Relative Humidity (RH) and Temperature-Humidity Index (THI). The results demonstrated that the month of semen collection had no effect (P>0.05) on VOL, CONC and TOT. However, MOT and STR were differed (P<0.05) among semen collection time, with the peak value was observed in March. Among climatic variables studied here, AT_{max} was the most sensitive factor associated with sperm quality parameters. AT_{max} value at the same month as semen collection had negative significant correlation (P<0.05) with TOT and STR, while at one and two months before semen collection was consistently had negative significant correlation (P<0.01) with CONC, TOT and STR. It could be concluded that the maximum ambient temperature is the most responsible climatic factor affecting loss of straw production in Madura bulls.

Keywords: Artificial Insemination, Beef Cattle, Indonesian Local Cattle, Semen Cryopreservation, Tropical Region

Introduction

Madura cattle have been recognized as one of the Indonesian native breeds (KMPN, 2010). This breed is a result of crossing between Bali cattle (Indonesian origin breed) and Zebu cattle (Indian origin breed) which occur approximately 1,500 years ago in Madura Island, Indonesia (Sutarno and Setyawan, 2016). Madura cattle have predominantly brick red of body color with the white color in legs and rump (Sutarno and Setyawan, 2015). In addition, they also have small and short horn led to the outside (Maylinda *et al*., 2017). The population of Madura cattle are mostly concentrated in Madura Island, Indonesia (Zuhri *et al*., 2019). Due to their good adaptability to the tropical environment, Madura cattle are expected could contribute significantly to fulfill national meat demand. However, it was reported that Madura cattle population was still remained low, which only contribute to about 5.16% of the total cattle population (Sutarno and Setyawan, 2015). For that reason, the use of advanced technology such as Artificial Insemination (AI) is necessary to improve the productivity of Madura cattle.

The good semen quality is a critical point to ensure the success of the AI program. While semen quality itself is highly dependent on climate factors such as Ambient Temperature (AT), Relative Humidity (RH), or their combination as Temperature-Humidity Index (THI)

(Snoj *et al*., 2013; Bhakat *et al*., 2014; Valeanu *et al*., 2015; Sabés-Alsina *et al*., 2017; Isnaini *et al*., 2019a; Isnaini *et al*., 2020; Isnaini *et al*., 2021a). Among those mentioned factors, AT seems to be the main variable which may affect semen quality. The increased in AT may induce heat stress, which strongly associated with the impairment of spermatogenesis and the reduction of sperm quality (Rahman *et al*., 2018). Since the duration of spermatogenesis in cattle is approximately 60 days, the deleterious effect of high AT is not sufficient to be observed only at the same time of semen collection (Sabés-Alsina *et al*., 2019). Currently, the information about the relationship between climatic variables and dynamic change of semen quality in Madura bulls is still very limited. Therefore, the present study was carried out to elaborate the association between climatic factors, either at the same month as, one month before, or two months before semen collection with fresh semen quality parameters in Madura bulls.

Materials and Methods

Experimental Site and Climatic Factors

This study was conducted at Lembang AI Center (Bandung, Indonesia), which was situated at 6.82°S 107.63°E. The elevation of this site is about 1,241 m above sea level. The data of mean and maximum ambient temperature (AT_{mean} and AT_{max}, °C) and relative humidity (RH, %) were obtained from Bandung Geophysics Station, which was located approximately 11 km from the bull station. The Temperature-Humidity Index (THI) were calculated with a formula of $THI = 0.8 \times AT_{mean} + (RH/100) \times (AT_{mean} - 14.4) + 46.4$.

Animals and Feeding

A total of five Madura bulls were included in the study. The age of bulls at the beginning of the study was ranged from two to four years. The selection of bull was carried out by taking into account the performance of Madura cattle based on the Indonesian National Standard (SNI 7651.2: 2013). The selected bull must also be in good health, free from infectious animal diseases and free from physical and reproductive organs defects. The semen quality of bulls also should meat the Indonesian National Standard (SNI 4869-1: 2017)

The bulls were reared with similar management practices under standard protocol in Lembang AI center. Each of the bull was kept in an individual cage. The bulls were provided with constant feeding during experimental period. The daily ration consisted of 40 kg elephant grass (*Pennisetum purpureum*), 1 kg African grass hay (*Cynodon plectostachyus*), 3 kg commercial concentrate feed and 300 g mung bean sprout (*Vigna radiata*). The commercial concentrate feed had 16% crude protein content and 65% total digestible nutrient. The drinking water was provided *ad libitum* throughout the study.

Semen Collection and Evaluation

The semen collection was conducted twice a week from January 2014 to December 2018 (n = 1583). Upon collection, the semen quality parameters were directly evaluated for semen Volume (VOL) using a scaled tube (Kusumawati *et al*., 2018). After that, sperm Concentration (CONC) was measured using a spectrophotometer (Isnaini *et al*., 2019b). Total sperm (TOT) was calculated by VOL x CONC (Isnaini *et al*., 2019c). Individual motility (MOT) was evaluated using a phase contrast microscope (Deonizio *et al*., 2018). The ejaculates with MOT >70% were included in the straw production (STR) calculation with a formula of TOT/25 million (Isnaini *et al*., 2019c).

Statistical Analysis

All statistical procedures were performed using SPSS ver. 13 software (SPSS Inc., Chicago, IL, USA). Data of semen quality were evaluated using analysis of variance with the month of semen collection as a factor. Duncan's multiple range test (Duncan, 1955) was employed to compare means, with statistical significant was set at $P<0.05$. Relationship between climatic factors and semen quality parameters were evaluated using Pearson correlation test (Pearson, 1931).

Results

Climatic Factors

Figure 1 shows the monthly climatic factors in the experimental site throughout the study. AT_{mean} was ranged from 22.9 to 25.3°C with variation coefficient of 2.05%. AT_{max} was ranged from 26.6 to 31.9°C with variation coefficient of 2.97%. AT_{max} was relatively higher in September and October, but relatively lower in January and February. RH was ranged from 62.6 to 83.8% with variation coefficient of 6.89%. THI was ranged from 70.4 to 75 with variation coefficient of 1.17%.

Semen Quality Parameters

Table 1 shows the semen quality parameters of Madura bulls from 2014 to 2018. The overall mean values of VOL, CONC, TOT, MOT and STR were 5.31 mL/ejaculate, 1.04 billion/mL, 5.43 billion/ejaculate, 63.9% and 169 unit/ejaculate, respectively. The month of semen collection did not affect ($P>0.05$) VOL, CONC and TOT, while MOT and STR were affected ($P<0.05$). The lowest MOT was recorded in July, while the higher value in March and December. In the case of STR, the lower value was observed in January, July, August and November, while the peak value in March.

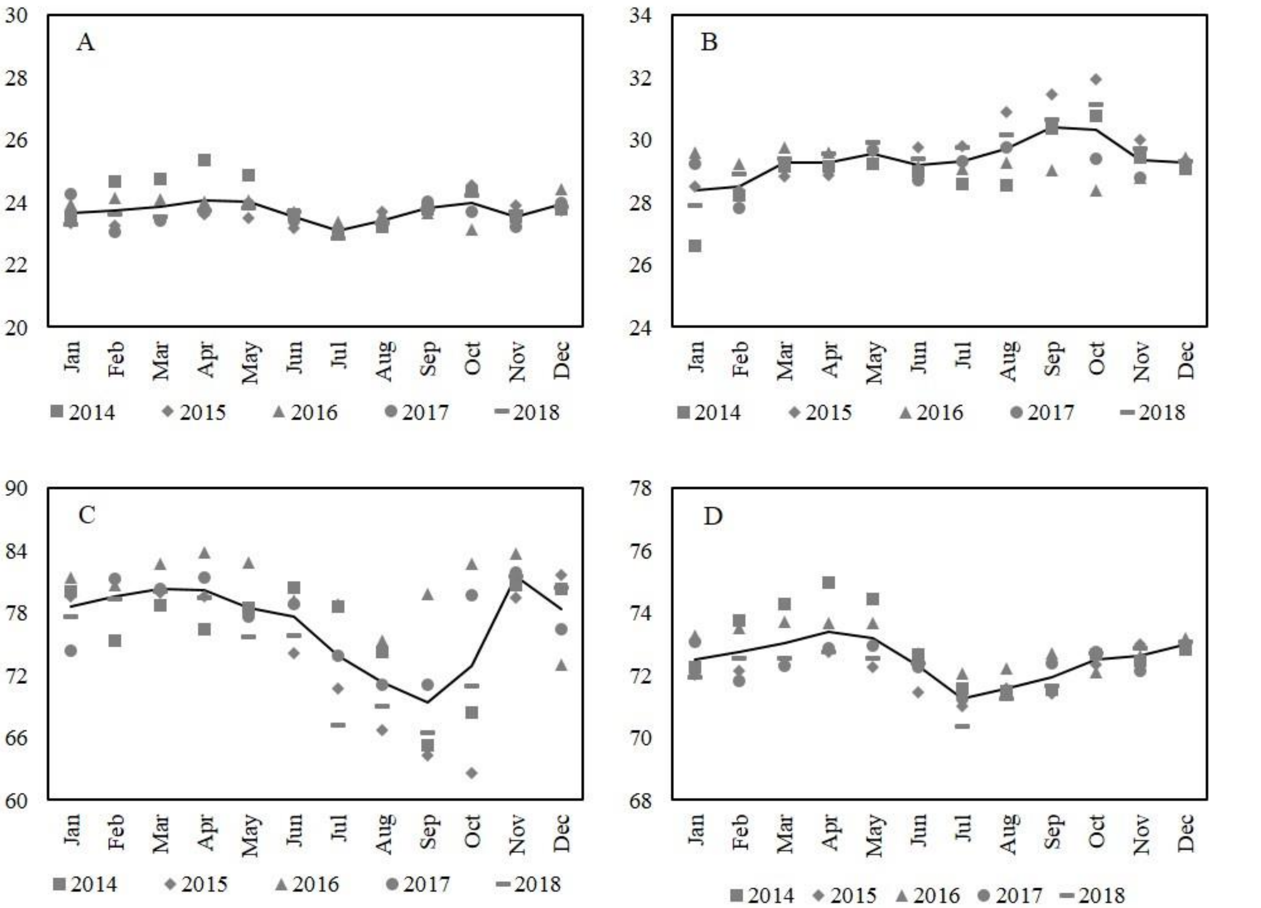

Fig. 1: Monthly climatic factors from January 2014 to December 2018 in Bandung, Indonesia. A: Mean ambient temperature (AT_{mean},°C), B: Maximum ambient temperature (AT_{max},°C), C: Relative Humidity (RH, %), D: Temperature-humidity index (THI). (-) Black line inside the graph represents mean values from 2014 to 2018.

Table 1: Summary of semen quality parameters (mean ± SE) of Madura bulls

Month	n	VOL	CONC	TOT	MOT	STR
January	141.00	5.09±0.14	0.96±0.03	4.9±0.19	62.9±1.18[abcd]	151±10.4[a]
February	120.00	5.58±0.18	1.06±0.03	5.75±0.21	65.9±0.89[cd]	194±11.5[bc]
March	145.00	5.46±0.13	1.08±0.03	5.79±0.18	66.1±0.86[d]	196±9.92[c]
April	111.00	5.24±0.15	1.04±0.03	5.38±0.22	64.2±1.1[abcd]	171±12.2[abc]
May	104.00	5.68±0.18	1.04±0.03	5.76±0.2	65.4±1.06[cd]	189±11.4[bc]
June	118.00	5.43±0.16	1.04±0.03	5.56±0.21	61.7±1.22[ab]	161±12.4[ab]
July	147.00	5.22±0.15	1.13±0.09	5.85±0.49	61.3±1.17[a]	152±10.3[a]
August	163.00	5.18±0.13	1.04±0.02	5.28±0.16	62.5±1.02[abc]	153±9.97[a]
September	122.00	5.01±0.13	1.07±0.03	5.28±0.17	64.9±0.9[bcd]	160±10.9[ab]
October	158.00	5.32±0.15	1±0.03	5.33±0.2	64.2±0.99[abcd]	168±10.4[abc]
November	125.00	5.23±0.15	0.98±0.03	5.06±0.2	62.8±1.23[abcd]	153±10.7[a]
December	129.00	5.41±0.14	1±0.03	5.36±0.18	66±0.99[d]	184±9.99[abc]
P value		0.08	0.113	0.083	0.002	0.003

n: Number of ejaculates, VOL: Semen volume (ml/ejaculate), CONC: Sperm concentration (billion/ml), TOT: Total sperm (billion/ejaculate), MOT: Individual motility (%), STR: Straw production (unit/ejaculate)
[a-d]different superscript letters denote significant difference among months of semen collection ($P<0.05$)

Table 2: Relationship between climatic factors and semen quality parameters

Items	VOL	CONC	TOT	MOT	STR
Same month as semen collection					
AT_{mean}	-0.01	-0.05	-0.05	0.05*	0.01
AT_{max}	-0.05	-0.03	-0.05*	0.01	-0.05*
RH	0.1**	-0.02	0.05	0.03	0.08**
THI	0.06*	-0.05*	-0.01	0.06*	0.06*
One month before semen collection					
AT_{mean}	-0.01	-0.02	-0.02	-0.03	-0.03
AT_{max}	-0.05	-0.08**	-0.09**	-0.03	-0.09**
RH	0.09**	0.04	0.08**	0.02	0.08**
THI	0.05*	0.002	0.04	-0.02	0.02
Two month before semen collection					
AT_{mean}	-0.04	0.02	-0.002	-0.02	-0.02
AT_{max}	-0.03	-0.07**	-0.07**	-0.03	-0.07**
RH	0.06*	0.05*	0.07*	0.02	0.04
THI	0.01	0.05*	0.05	-0.04	0.01

*Significant correlation at P<0.05, **Significant correlation at P<0.01

Relationship between Climatic Factors and Semen Quality Parameters

Table 2 shows that there were several significant correlations between climatic factors at the same month as semen collection with semen quality parameters. The positive correlations were found on AT_{mean} with MOT (P<0.05), RH with VOL and STR (P<0.01) and THI with VOL, MOT and STR (P<0.05), whereas, the negative correlations were found on AT_{max} with TOT and STR (P<0.05), as well as THI with CONC (P<0.05). There were also several significant correlations between climatic factors at one month before semen collection with semen quality parameters. RH had positive correlations with VOL, TOT and STR (P<0.01). THI also positively correlated with VOL (P<0.05). On the other hand, AT_{max} was negatively correlated with CONC, TOT and STR (P<0.01). In addition, several significant correlations between climatic factors at two months before semen collection with semen quality parameters were also detected in this study. At this time point, the positive correlations were found on RH with VOL, CONC and TOT (P<0.05) and THI with CONC (P<0.05). While, the negative correlations were found on AT_{max} with CONC, TOT and STR (P<0.01).

Discussion

In this study, the climatic variables could be categorized as stable due to the low variation coefficient value throughout the experiment. It was also previously reported that in Semarang, Indonesia (another location of Indonesian AI center), the AT_{mean} and RH were almost equal throughout the year, with 26.6°C and 82.3% in rainy season and 26.9°C and 81.5% in dry season (Isnaini *et al*., 2019d), In another study, Brito *et al*. (2002) reported that the average AT_{mean} and RH in a Brazilian AI center were ranged from 19.5 to 25.7°C (mean = 23.4°C) and 60.9 to 81.4% (mean = 71.4%).

The overall mean values of sperm quality parameters obtained in this study were almost comparable to those reported by Ratnawati *et al*. (2018), who observed that the VOL, CONC, MOT of Madura bulls were 5.7 mL/ejaculate, 1.08 billion/mL and 66%. In comparison with other Indonesian native breeds, Madura bulls used in this study relatively had higher VOL but with lower CONC than Ongole Grade and Bali bulls (Kusumawati *et al*., 2017; Susilawati *et al*., 2018). Whereas, Madura bulls relatively had lower VOL but with higher CONC when compared with Pasundan bulls (Isnaini *et al*., 2021b).

No difference among semen collection time toward VOL, CONC and TOT was consistent with previous findings. Brito *et al*. (2002) noted that there was no significant effect of the month on VOL, CONC and TOT of breeding bulls raised in the tropical area of Brazil with only less than 2% of variation among the months. Similarly, Isnaini *et al*. (2019d) also found that VOL, CONC and TOT of Simmental bulls were remained unchanged during rainy and dry seasons. In another study, Landaeta-Hernández *et al*. (2020) also reported that the season of collection had no significant effect on CONC of crossbred bulls. Although the variation in MOT and STR exists in the present finding, the semen collection is still acceptable to be conducted throughout the year. In the case of the peak STR which is achieved in March, this evidence probably as a reflection of the lower AT_{max} in January and February, which allow more ideal condition for spermatogenesis. On the other hand, when AT_{max} reaching its high points, such as in September and October, the reduction in STR is observed in November.

This current study demonstrated that AT_{max} represents as the most sensitive factor associated with sperm quality parameters. AT_{max} value at all-time points consistently had negative significant correlations with TOT and STR. It could be explained that the increased in environmental temperature led to the increased in scrotal temperature, which even in only a small degree or at a short period, could generate oxidative stress (Alves *et al*., 2016; Boe-Hansen *et al*., 2020). The oxidative stress then may interfere productive and reproductive response, as well as decreasing TOT (Brito *et al*., 2003; Nichi *et al*., 2006; Andri *et al*., 2016; De Ruediger *et al*., 2016; Andri *et al*., 2018). Since TOT data are used as the basis of STR calculation, their reduction will be followed by the latter.

In this study, the higher correlation value (r) between AT_{max} with CONC, TOT and STR were detected at one month before semen collection, which indicates that the spermatogenesis stage at this time point is more susceptible to heat stress rather than at the same month as or two months before semen collection. This result probably due to the adverse effect of heat stress was more pronounced at spermiogenesis and meiosis phases, as previously elaborated by Rahman *et al.* (2011) that the peak proportion of sperm with low protamine content due to the heat stress induction was found at days 28 to 35 of spermatogenesis. The low protamine content could contribute to the DNA damage (Fortes *et al*., 2014), which may further be associated with the decreased in CONC (Schulte *et al*., 2010) and consequently will be followed by the reduction in TOT and STR.

Conclusion

In conclusion, the maximum ambient temperature is the most responsible climatic factor affecting loss of straw production in Madura bulls.

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Author's Contributions

Nurul Isnaini: Conception and design, analysis and interpretation of data, reviewing manuscript critically for significant intellectual content and give final approval of the version to be submitted and any revised version.

Bela Dwi Wadi Lestari and Faizal Andri: Conception and design, acquisition of data, analysis and interpretation of data, drafting the manuscript and give final approval of the version to be submitted and any revised version.

Tri Harsi and Eros Sukmawati: Conception and design, acquisition of data, reviewing manuscript critically for significant intellectual content and give final approval of the version to be submitted and any revised version.

Ethics

The authors declare that there are no ethical issues may arise after the publication of this study.

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